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Title

Seroprevalence of anti-SARS-CoV-2 IgG antibodies: relationship with COVID-19 diagnosis, symptoms, smoking and method of transmission

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HIGHLIGHTS

- The most discriminative symptom of COVID-19 is by far the loss of smell/taste.
- In smokers, the incidence of positive COVID-19 diagnosis was significantly lower.
- There is a discrepancy between COVID-19 diagnosis and presence IgG antibodies.

Abstract

Aims: The study of SARS-CoV-2 antibodies in the population is a crucial step to overcome the COVID- 19. Seroepidemiological studies would allow to estimate the

number of people who have been exposed to the virus, as well as to estimate the number of people who are still susceptible to infection.

Methods: A total of 13.560 people from Arganda del Rey, Madrid (Spain), were assessed between January and March 2021 for the presence of IgG antibodies using rapid tests and history of symptoms compatible with COVID-19.

Results: We found that 24,2% of the participants had IgG antibodies and 9% a positive COVID-19 diagnosis. Loss of smell/taste was the most discriminative symptom of the disease. Main transmitters of infection were mostly household member. Unexpectedly, in smokers, the incidence of positive COVID-19 diagnosis was significantly lower. Also, it was found that there was a discrepancy between the diagnosed cases and presence of IgG antibodies and between those that showed antibodies and COVID-19 diagnosis.

Conclusions: Rapid anti-IgG test are less reliable to detect SARS-CoV-2 infection at an individual level but are functional to estimate SARS-CoV-2 infection rate at an epidemiological level. Also, the loss of smell/test is a potential indicator to estimate COVID-19 infection.

Key Words: COVID-19, IgG antibody, seroprevalence, smoking, smell/taste

Introduction

Since the beginning of the COVID-19 pandemic, a large number of seroepidemiological studies have been conducted in order to determine the presence of antibodies against SARS-CoV-2 (Strinhgini et al., 2020; Eslami & Jalili, 2020; Mack et al., 2021; Figueiredo-Campos et al., 2020). There are two main premises of these studies. The first is that the presence of antibodies allows, in an objective way and despite being asymptomatic

cases, to estimate the number of people who have been exposed to the virus. The second is to be able to estimate the number of people who are still susceptible to infection. This is important to determine whether the threshold of herd immunity has been reached. The concept of herd immunity refers to the fact that a large part of the population must have been exposed to the virus naturally (infected) or by vaccination. Herd immunity has been estimated at 50-66% in the SARS-CoV-2 pandemic (Neagu, 2020). This would result in a lower probability of infection between individuals.

An added value of the seroepidemiological studies are the self-reports that can accompany the clinical assessment of IgG antibodies. Thanks to these questionnaires it is possible to determine which symptoms have greater predictive value for the diagnosis of COVID-19. On one hand, serologic testing can detect cases that have been asymptomatic (Arabkhazaeli et al., 2021; Shakiba et al., 2020). On the other hand, the patients' self-report data allow us to associate these symptoms with the test results. For example, one of the symptoms that appears to be most associated with SARS-CoV-2 infection is loss of taste and/or smell (Marcgese-Ragona et al., 2020; Aziz et al., 2021). There is also a debate between smoking activity and the likelihood of worsening COVID-19 disease course, and/or number of hospital admissions in the smoking population (Vardavas & Nikitara, 2020; Farsalinos, Barbouni & Niaura, 2020; Proppers, 2020; Gupta et al., 2021). Intuitively, one would tend to think that in the smoking population the number of COVID-19 cases would be higher. In addition, epidemiological studies allow us to explore the main routes of infection. It is to be expected that proximity, cohabitation between individuals, and the duration of cohabitation are the main factors facilitating contagion.

For this study we have relied on the national seroepidemiological study in Spain that has been a reference for the Spanish population and published in the journal The lancet in July 2020 (Pollán et al., 2020). We have adapted most of the methodology used in this national study to a local study in a population of Madrid. To increase the extrapolability/comparability between both studies we have used the same serological rapid test and replicated most of the items of the self-reported questionnaires applied to the participants.

Material and Methods

Study design and participants

The study design included three successive stages of data collection, with a three-week break between each one of them: from January 18th to 23rd, from February 15th to 20th and from March 15th to 18th, 2021. Participants were randomly selected based on the town census of Arganda del Rey. In order to have participants of a wide age range, the selection was made by household. All household residents were invited to participate in the study, resulting in a final sample of 13,560 individuals, representing 24,04% of the entire population of Arganda del Rey (National Institute of Statistics, 2021). The main characteristics of the sample of participants are described in Table 1.

Individuals residing in the selected households were contacted by telephone and informed of the objective and characteristics of the study. Once their willingness to participate had been confirmed, participant information was obtained and an appointment for the rapid immunochromatographic test was made at the facilities provided by the City Council of Arganda del Rey.

The telephone contact service was provided by the company "CTi Soluciones". The inclusion criteria for the study were: (1) being registered in the town of Arganda del Rey, (2) being older than one year of age, (3) having knowledge of the Spanish language.

Written informed consent was obtained from all study participants. Different forms of informed consent were used for adults, teenagers, parents of participating children, and guardians of mentally disabled participants. All data collected for the study were identified by a random code to protect the identity of the participants. The study complied with Organic Law 3/2018 of December 5, 2018, on Personal Data Protection and Guarantee of Digital Rights, and Regulation (EU) 2016/679 of the European Parliament and of the Council of 27 April 2016 on Data Protection (General Data Protection Regulation - GDPR). The study obtained the favorable opinion of the Research Ethics Committee of the Community of Madrid (Law 14/2007 on Biomedical Research).

General Procedure

Once contacted and invited, the participants attended the town sports center "Príncipe Felipe", where they filled out the informed consent form and answered a questionnaire that included history of symptoms compatible with COVID-19 (i.e., fever, chills, fatigue, sore throat, cough, shortness of breath, headache and loss of taste or smell, among others), contact with suspected or confirmed cases, and other risk factors. The epidemiological questionnaires were applied by professional psychologists contracted through the Complutense University of Madrid. Blood samples were then extracted by finger prick. These extractions were performed by 16 nursing

professionals. Once the extraction was completed, the participants left the facilities and the samples were sent to the Faculty of Medicine of the Complutense University of Madrid, where they were centrifuged and the rapid immunochromatographic test was performed. Transport of samples to the laboratories was carried out in accordance with current regulations for the transport of category B infectious substances (packing instruction P650 and UN3373). Disposal of biological waste was carried out according to the regulations described in the BOE of March 22, 2020.

Detection of SARS-CoV-2 antibodies

The analysis of the SARS-CoV-2 antibodies was carried out by means of a rapid immunochromatographic test (Orient Gene Biotech COVID-19 IgG/IgM Rapid Test Cassette; Zhejiang Orient Gene Biotech, Zhejiang, China), a lateral-flow immunochromatographic assay for qualitative differentiation between IgG and IgM against the receptor binding domain of SARS-CoV-2 spike (S) protein, which yields results in 10 min. The manufacturer reported sensitivity of 97.2% and specificity of 100% for IgG, using RT-PCR as the gold standard. The used antibody tests have been shown in previous quality studies, to archive indeed high sensitivity and specificity (Hanssen et al., 2021) and have been also used previously in several seroprevalence studies (Akpabio et al., 2021; Álvarez-Antonio et al., 2021). Blood samples were extracted by finger prick and centrifuged in order to detect SARS-CoV-2 antibodies in serum samples. Due to the lower sensitivity and specificity of IgM, its shorter duration, and the heterogeneity of results observed in initial IgM readings, results for the point-of-care test reported here are based only on IgG.

Statistical Analysis

In this study, a total of 13.560 individuals from Arganda del Rey, Madrid, Spain were assessed for the presence of IgG antibodies. All the questionnaires were checked manually and exported to SPSS version 23 software package (SPSS Inc., Chicago, IL, USA) for analysis. After performing descriptive analysis, direction, and strength of statistical association between symptoms and COVID-19 diagnosis or presence of IgG antibodies were measured by odds ratio with 95% CI. In this study p value <0.05 was considered to declare a result as a statistically significant association.

Results

Relationship between symptoms, presence of a positive COVID-19 diagnosis and IgG antibodies

Considering the total population data (N=13,560), about one in ten participants had a positive COVID-19 diagnosis (9% / n= 1,224), and about one in four participants showed IgG antibodies (24.2% / n=3,286). Among the population that did not have IgG antibodies, 97% did not have a positive COVID-19 diagnosis. Among the population with a positive COVID-19 diagnosis, 74.5% had IgG antibodies. Among the population without a positive COVID-19 diagnosis, 19.2% had IgG antibodies.

The symptoms associated with a positive COVID-19 diagnosis were, from most to least frequent: headache (38.4%), fatigue (27.4%), cough (24.2%), loss of smell/taste (24.1%), sore throat (19.6%), chills (17.9%) and fever (17.1%). However, the higher frequency of a symptom should not be confused with that symptom being the most discriminative of having COVID-19. As can be seen in Figure 1, the symptoms of loss of smell and fever are those that are not found in most of the population without a COVID-19 diagnosis, 1.5% and 2.7%, respectively. These would be the symptoms that

most discriminate and support a differential diagnosis. Similar results are obtained exploring the association between symptoms and presence of IgG antibodies.

Exploring the association between more common symptoms and common disease included in the questionnaire and a positive COVID-19 diagnosis (Figure 2 panel a) and the presence of IgG antibodies (Figure 2 panel b), the association between loss of smell/taste and COVID-19 diagnosis is again extremely significant. In this way, the association between loss of taste/smell and COVID-19 diagnosis, arises OR 21.43 (95% CI: 17.50 - 26.25). This suggests that this symptom is more than 21 times more likely to be found in the COVID-19 population than in the control population. Other symptoms also all show statistically significant ORs, albeit in a more modest fashion.

None of the evaluated disease, including diabetes, arterial hypertension, cardiovascular disease, pulmonary disease, and a general section of other chronic diseases, showed any association with COVID-19. This indicates that the presence of COVID-19 was independent of any of these diseases.

Relationship between being a smoker and having had a positive diagnosis of COVID-19 and IgG antibodies.

Approximately one in four study participants were smokers (28.4% / n=3,768).

Considering the entire sample, among the smoking population there was a higher percentage of participants who did not have a positive diagnosis for COVID-19 (29%) vs. 21.7% of smokers who did have a positive diagnosis. Figure 3 shows the percentage of smokers who claimed to have or not to have a positive COVID-19 diagnosis as well as the presence of IgG antibodies in both populations. As shown, smokers the probability of being diagnosed with COVID-19 was 33.6% lower. Also, the frequency of

the presence of IgG antibodies differs between smokers and non-smokers. Whereas 26,6% of non-smokers showed IgG antibodies, these value decreased significantly to 19,26 % in smokers ($\chi 2$ (1)=70,36 ($p<0.001$)).

Transmission of COVID-19 and relationship to presence of IgG antibodies

Only 4.6% of the participants who had a positive diagnosis state that they had no contact with anyone diagnosed with COVID-19, or at least that they were aware of, whereas the most frequent source of identified infection was a member of the household, followed by a customer or patient and non-cohabiting relative or friend (Figure 4 panel a). When evaluating the relationship between having been in contact with a person diagnosed with COVID-19 and the presence of IgG antibodies, the results follow the same pattern. Here 19.4% of the participants with IgG antibodies stated that they had no contact with anyone diagnosed with COVID-19, or at least to their knowledge. Again, contact with infected customers (including patients) and cohabiting persons is associated with higher level of IgG presence.

Discussion

The main findings of the study to be discussed below are the following: among the symptoms associated with COVID-19, loss of smell/taste is the most discriminative symptom of the disease. In smokers, the incidence of positive COVID-19 diagnosis is significantly lower. Most of those diagnosed with COVID-19 or who had IgG antibodies had identified the person who could have infected them, being the most frequent infection by a member of the household. The use of rapid IgG serological tests for the detection of SARS-CoV-2 virus is an effective tool for epidemiological studies of populations, despite its deviations in individual measurements.

Currently, at the time of writing this paper, there are several reviews stating that most patients who contract COVID-19 are asymptomatic or have mild to moderate symptoms of the disease. For example, among the population under twenty years of age between 15-42% are asymptomatic (Viner et al., 2020). It is interesting to note that loss of smell and taste were among the first symptoms to be seen associated with COVID-19 (Russell et al., 2020; Lorenzo Villalba et al., 2020). One of the first studies to propose the usefullness of hyposmia and hypogeusia for the diagnosis of COVID-19 was the paper of Bénézit et al. (2020). In that article, with a sample of 68 patients with COVID-19 and 189 patients without the disease they found Odds Ratios between 7.44 - 13.44. In our case, with a sample of 1,224 participants with a positive COVID-19 diagnosis and 12,336 control participants we found a significantly higher Odds Ratio: 21.43 (95% CI: 17.50 - 26.25). Therefore, our results would support the proposal of the usefulness of the lack of taste and smell as one of the predictive tools in the diagnosis of COVID-19 in case of impossibility of clinical laboratory tests, such as RT-PCR or rapid antigen tests. Other frequent symptoms of COVID-19 are also as frequent as in other types of diseases or infectious processes that they offer a lower capacity for differential diagnosis. Moreover taking into account the presence of IgG antibodies as a witness of having been exposed to the SARS-CoV-2 virus, we see that loss of smell and taste are those most specifically associated with the presence of IgG antibodies.

One of the most controversial results of our study is the relationship between smoking and fewer positive COVID-19 diagnosis. As early as March 2020, a first systematic review was published which concluded, that smoking was associated with worse progression and negative consequences of COVID-19 (Vardavas & Nikitara, 2020). New research continues to point in the same direction. It appears that smoking is associated

with increased risk of having severe COVID-19 symptoms in hospitalized patients (Saadatian-Elahi, 2021). However, another set of studies shows different results. For example, the prevalence of hospitalized COVID-19 smokers in China has been shown to be 10.2% when the expected prevalence would be 31.3%. This would go against the argument that being a smoker is a risk factor for hospitalization for COVID-19 (Farsalinos, Barbouni & Niaura, 2020; Propper et al., 2020). In our study 28.4% were smokers. Therefore, if smoking activity were independent of the number of positive COVID-19 diagnoses, we should have found approximately 28-30% of smoking participants diagnosed with the disease. However, the case of positives in smokers was reduced to 21.7%. Our data support the idea that the probability of a positive COVID-19 diagnosis, and possible hospitalization of the patient, is significantly lower in the smoking population. It seems that angiotensin-converting enzyme 2 (ACE-2) has a key role in susceptibility to the virus and smoking, but studies still show controversy (Gupta et al., 2021). As a conclusion, it seems that in smokers the rate of hospitalization or positive COVID-19 diagnosis is lower, but at the time they are hospitalized their prognosis is worse.

With regard to the association between being a smoker and the presence of IgG antibodies against the SARS-CoV-2 virus, the data show that 26,2% of non-smokers had IgG antibodies while only 19,29% of smokers did have antibodies. Certainly, several explanatory hypothesis can be proposed from this data. One of these hypothesis could be that smokers are impaired in their ability to produce IgG antibodies against SARS-CoV-2, since the percentage of smokers without antibodies was significantly higher than those with antibodies. However, given that the presence of a positive COVID-19 diagnosis was also taken into consideration, these data help to interpret that indeed

the presence of IgG antibodies serves as an indicator of the number of infections. Therefore, since smokers showed reduced IgG antibodies as well as a reduced frequency in COVID-19 diagnosis, both data in combination indicate an association between smoking and reduced virus infection. Also, this finding between IgG and COVID-19 diagnosis supports the purpose of using IgG antibody levels as a proxy in the general population for virus exposure.

The most frequent methods of transmission of the virus appears to be household members, clients (e.g., a patient in the case of healthcare workers) and family members or friend who did not reside in the same household. Therefore, these three factors represent the major route of transmission of the virus. Only 4.6% of the COVID-19 diagnosed population did not know from whom they had been infected, suggesting a high percentage of awareness in the population. These results agree with other epidemiological studies. Thomson and colleagues, in a recent systematic review and meta-analysis, have shown that the home was the focus of infection and that, if the duration of contact exceeded five days, the rate of transmission was significantly higher (Thompson et al., 2021). Evaluating IgG antibody levels and contact with a person with a positive COVID-19 diagnosis, one should expect to find percentages similar to those described above. But according to our data these percentages are higher specially in the case of participants that could not identify the source of infection. Here, 19.4% of the participants who showed IgG antibodies did not know who could have transmitted the virus to them. This is almost four times more than in the case of participants who had a positive COVID-19 diagnosis. This would suggest that some percent of people have been exposed to the virus and have not developed

COVID-19 symptoms or did not relate the experienced symptoms to a possible COVID-19 infection.

Regarding the extrapolation of the results shown here, the percentages of infections found here in this specific locality of Madrid are higher than those found in the Spanish national study carried out earlier by Pollán et al., (2020). For example, the IgG seroprevalence in Spain at the time of the study (April-May 2020) was 11.3% in the province of Madrid. By the time of our study in the Madrid town of Arganda del Rey, almost one year later (January-April 2021) the IgG seroprevalence was 24.2%, including people who had already been vaccinated (n=399 / 2.9%). They have not been discarded from the study because many of these participants had already been infected by the virus previously. Therefore, what our study suggests is that in the 10-12 month period between studies, the IgG seroprevalence rate in the Madrid population has increased by about 16.5%.

Population serological studies have been used mainly to estimate the prevalence of SARS-CoV-2 infection since in many cases COVID-19 is asymptomatic (Pollán et al., 2020; Le Vu et al., 2021). It has been estimated that 35.1% of those infected by the virus are asymptomatic (Sah et al., 2021). According to our data, self-reports from participants show that 9% had had a positive COVID-19 diagnosis. In this population of positives, 25.5% had no IgG antibodies. That is, these participants would not be detected as infected using serological tests. And yet, when we evaluated the 91% of participants who did not have a positive COVID-19 diagnosis, 19.2% had IgG antibodies. Therefore, as a rough estimation, if we integrate the 25.5% of non-detected and the 19.2% of detected, it would compensate between both their

deviations. It is not an optimal solution but functional in terms of epidemiological estimation. Therefore, we could affirm that serological studies can indeed be a valid tool for establishing the prevalence of infection by the virus at the population level, but not at the individual level, since the estimated margin of error would be between 19-26%. Adding that these IgG antibody evaluations were performed with rapid tests, which had been previously validated clinically, our study suggests that rapid tests for antibodies against SARS-CoV-2 virus can help in an efficient and cost-effective way in population serological studies. At the time of the study, it could be ruled out that herd immunity had been achieved.

Ethical statement

The General Directorate of Public Health of Madrid approved this study (Project number: AR-COVID19). Informed written consent was obtained from all participants.

Author Contribution

EG and JALM were responsible for the conception, design, and coordination of the study. VEA, JCC, PDG, LSG were responsible for test analysis and acquisition of laboratory data. ADI, PMA and FGR were responsible for clinical revision of the study. MPW and JAM were in charge of statistical analyses and table and figure design. JALM, KMB and JCC wrote the first draft. All remaining authors contributed to data acquisition, laboratory analysis, interpretation and critically reviewed the first draft. All authors approved the final version and agreed to be accountable for the work.

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Conflict of interest

The authors declare no commercial or financial conflict of interest

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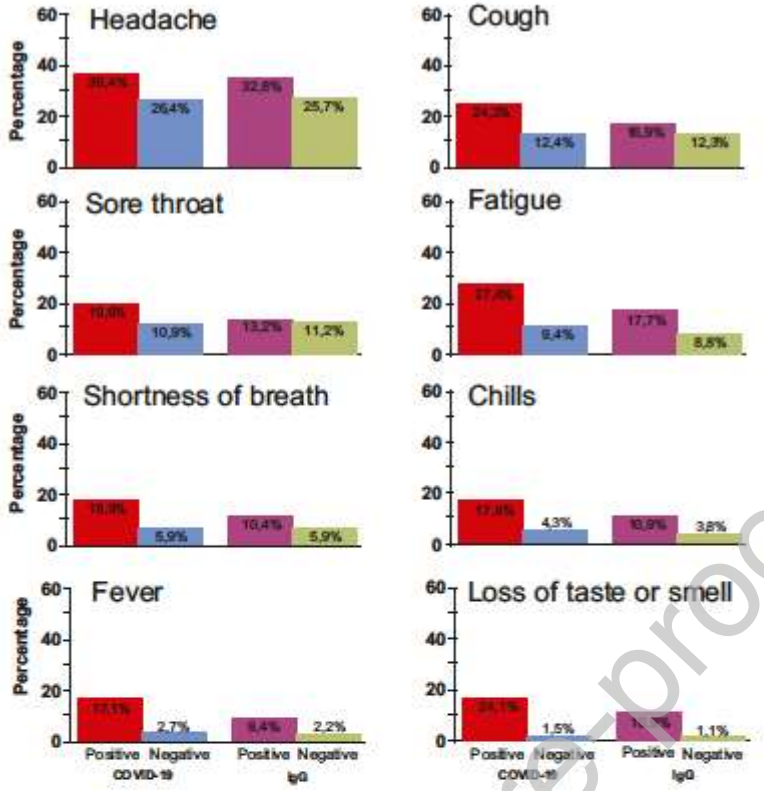
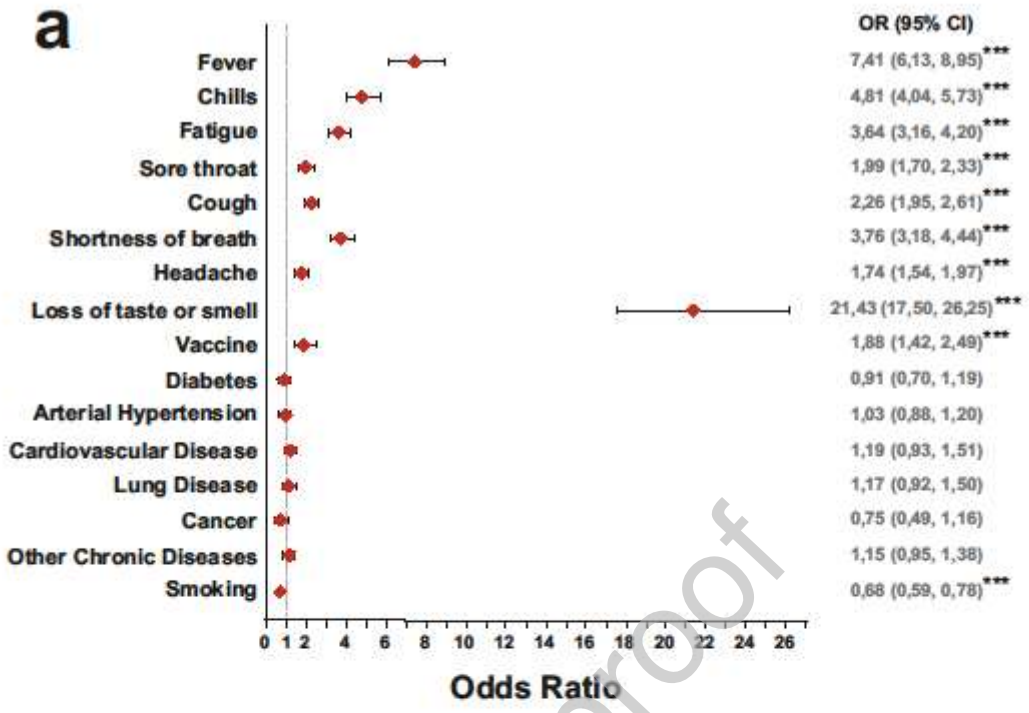


Figure 1. Association between symptoms and either positive COVID-19 diagnosis or presence of IgG antibodies. Bars on the **left** (red and blue colors) show the frequency percentages of

certain symptoms grouped by the presence of a positive or without COVID-19 diagnosis. Bars on the **right** (green and purple colors) show the association between symptoms and the presence or absence of IgG antibodies. The larger the orange/purple area indicates the more specific that symptom is to COVID-19 as it is less present in the general population.



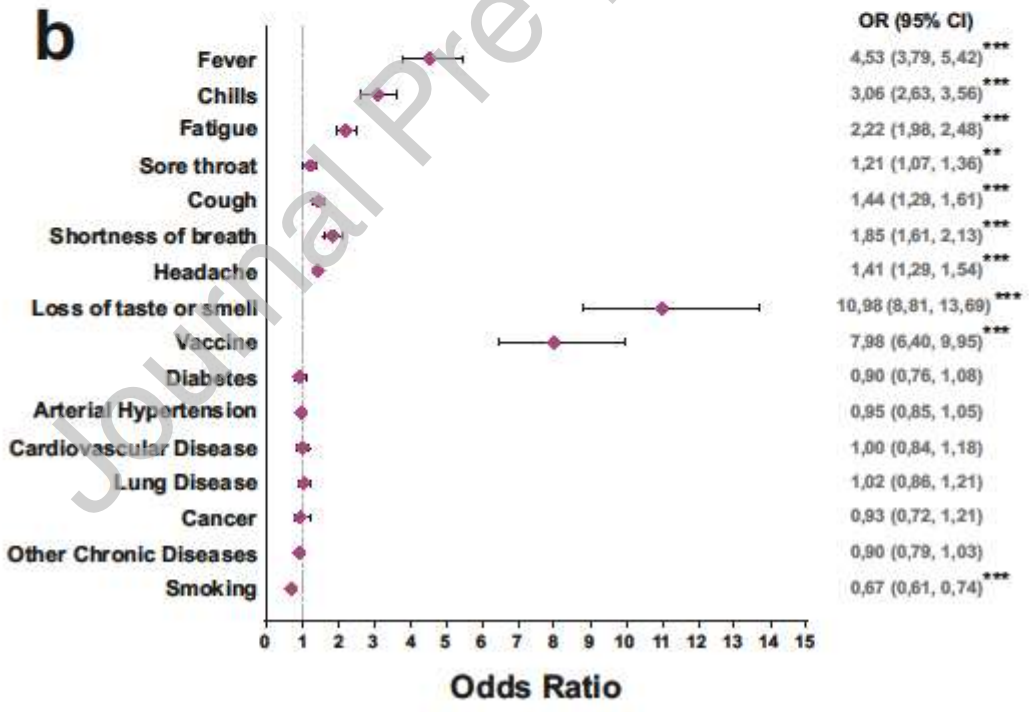


Figure 2. Odds Ratio (OR) and 95% confidence interval (95% CI) for COVID-19 symptoms and other common diseases associated with the presence of a positive COVID-19 diagnosis **(a)** and the presence of IgG antibodies **(b)**. In both cases, the most significant association was shown by loss of smell/taste, followed by fever. ** $p<0.01$; *** $p< 0.001$.

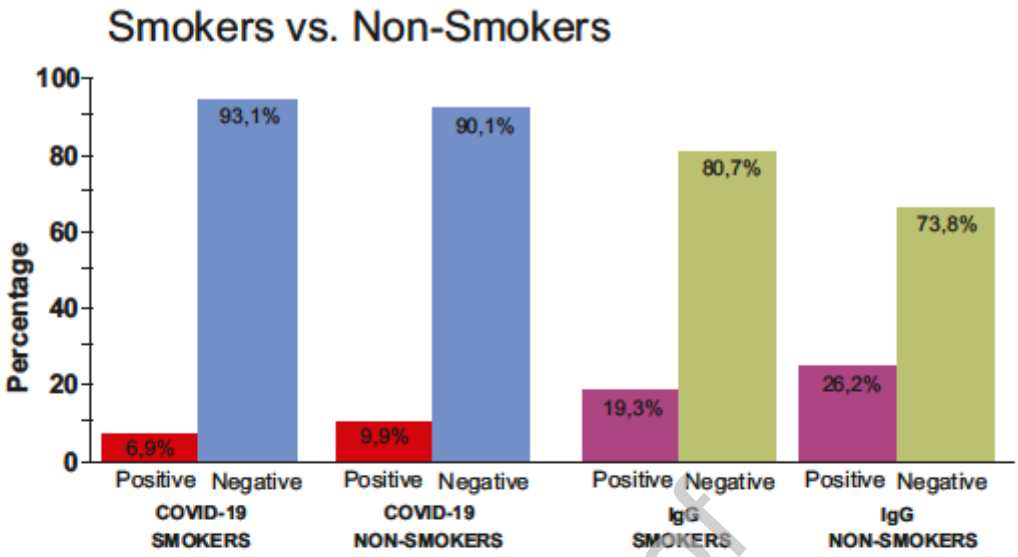
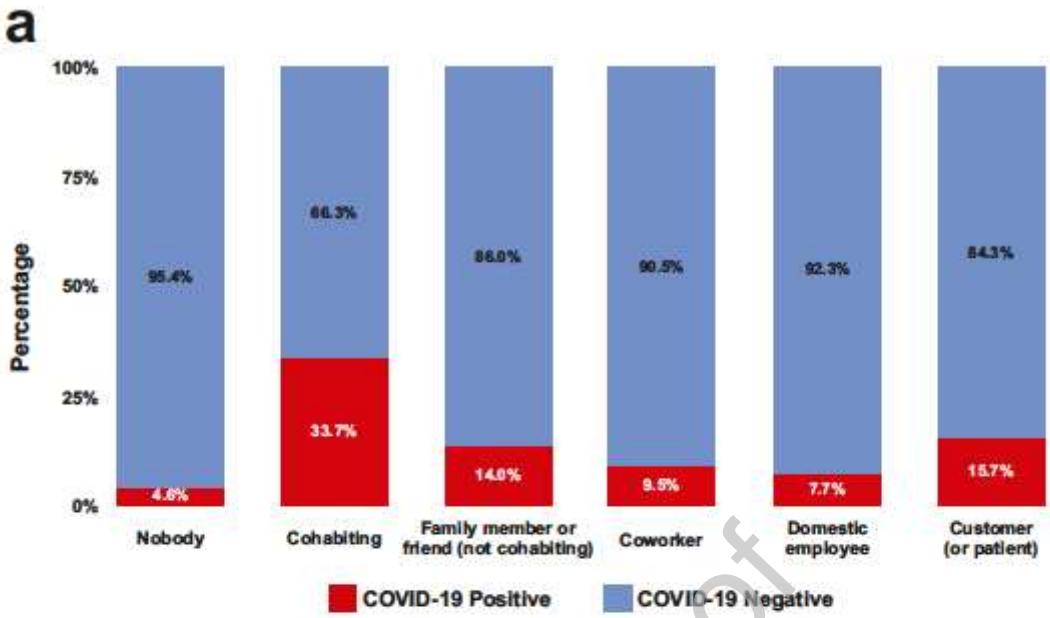


Figure 3. Association between smoking status and having a positive COVID-19 diagnosis and IgG antibodies presence. Among the smoking population, there was a higher percentage of participants who did not have a positive diagnosis for COVID-19 nor IgG antibodies. The association between smoking status and presence of IgG was significant at $p<0.001$ ($\chi 2$ (1)=70,36 ($p<0.001$))



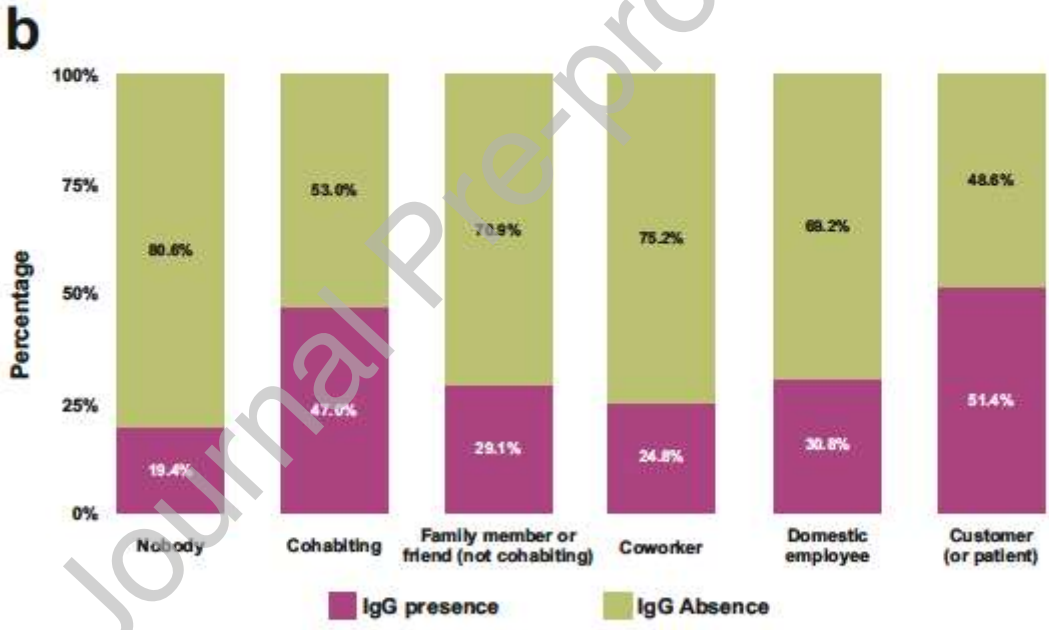


figure 4. Panel a shows the relationship between having been in contact with a person diagnosed with COVID-19 and the participant having had a positive diagnosis for the disease. **Panel b** shows the relationship between having been in contact with a person diagnosed with COVID-19 and the presence of IgG antibodies. The highest percentages of the presence of antibodies vs diagnoses indicates that a relevant number of participants have been exposed to the virus did not develop symptoms or did not have a positive diagnosis of COVID19

	Number of participants	Percentage (%)
Data collection stage		
1st Stage	4466	32,9
2nd Stage	5136	37,9
3rd Stage	3958	29,2
Sex		
Female	6289	46,4
Male	7271	53,6
Age, Years		
0-19	583	4,3
20-34	2216	16,3
35-49	5158	38,0
50-64	3433	25,3
≥65	2170	16,0
Nationality		
Spanish	11819	87,2
Other	1741	12,8
Occupation		
Active worker	7460	56,5
Retired	2217	16,8
Unemployed	1378	10,4
Student	987	7,5
House person	518	3,9
Other	633	4,8
Presence of COVID-19 at some point		
Positive COVID-19 diagnosis	1224	9,0
Negative COVID-19 diagnosis	12336	91,0
Vaccine		
Vaccinated participant -1º Dose	390	2,9
Vaccinated participant -2º Dose	139	1,02

Tables

Table 1. General characteristics of study participants